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INFLUENCE OF MICROORGANISMS COMMUNITY STRUCTURE ON THE RATE OF METALS PERCOLATION IN SOIL

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Abstract

A multidisciplinary international research was developed and field operation team was assembled to find opportunities to exploit bioremediation technologies for remediation of contaminated soil and ground water. In addition to standard chemical and hydrogeochemical analyses and laboratory based microbiological evaluations, potential toxicity constrains to bioremediation were assessed through the use of lab-pots (with low costs and controlled conditions) as well as ex-situ lysimeters experiments. They present an intermediate solution between laboratory and field test.

The aim of this research was to reproduce the circumstances where contaminants in the upper part of the soil are transported by rainfall and added to the pollutant in the deeper part. The soil was completely characterized from the physical and chemical point of view. Lab-pots experiments and a series of 35 small-lysimeters were performed using soil from three different heavy metal polluted areas, in order to assess the effect of mycorrhization and green fertilizer application on plants stressed by metals and quantity and quality of leachate.

Keywords: Bioremediation, Heavy metals; Lysimeters, VAM

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Introduction

In the terrestrial heavily polluted ecosystems with heavy metals there is a usually practice to use plants for (phyto) stabilising the surface of soil, practice used for reducing the export of metals through run-off or erosion and/or for reducing a part of metal stocks in soil as a results of the transfer of metals in plants, the phenomena being known as phytoextraction (Salt at al., 1998). However, not always it is preferable to perform a phytoextraction. When the metals are relative immobile in the redox conditions of terrestrial system, it is possible to be more suitable a phytostabilisation. The experiments shows that the calcium phosphat decrease the concentration of bivalent metals (Kucharski at. al, 2005), and from economical point of view the most advantageous is to perform the phytostabilisation with culture plants, usable in foods or industrial processes. The generic term for the possible using of plants in the heavily polluted ecosystems is the phytoremediation technique.

In the phytoremediation domain besides the obtaining of transgenic plants with accumulation and/or heavy metals very high resistance capacities, for instance the tolerance of tobacco transgenic plants by the cistein syntheses expression in citosol it was investigated for metals such Cd, Se, Ni, Pb and Cu. The transgenic tobacco plants were significantly more tolerant than the non transgenic ones on agar medium which contained Cd, Se and Ni (Kawashima at al., 2004). One of the actually research concern is the manipulation of bacteria and fungi in the rizosphere. If in the first instance the use of these organisms for the polluted soil remediation was limited to the organic contaminants (Salt et al. 1998), subsequent it was also extended to the heavy metals (Kothe, 2001, Iordache et al., 2004, Neagoe at al. 2004, 2005, and 2006). It is considered that the inoculation of soil with fungi immobilized in a certain measure the heavy metals from soil and encourages the development of plants. Such an effect is desirable especially in the case in which what is of interest is not the phytoextraction rather the phytostabilisation.

The mechanisms through the beneficial effect of inoculation which should be performed are very complex. Hairy roots of plants can influence the bioavailability and uptakes of metals from soil through alter of some parameters at the rizosphere level: lower decrease of pH, the increase of amount organic molecules (through exudation). Dissolved organic carbon play a key role both in the understanding of processes at the rizosphere level and regarding to the metals mobilise and immobilize mechanisms (Alloway, 1995, Scott, 2001). Bacterias from the rizosphere and fungi which form the mycorrhiza depends first by the organic dissolved exuded carbon and only in small measure by those resulted from the decomposition of dead organic matter (Harley and Smith, 1983). Some of the organisms from the rizosphere produced the siderophore molecules which are able to immobilize the metal, and which should have a role in both plants and organism protection by the toxic effect of metals (Ross, 1994). Other plant protection mechanism is to bound of metals to the hiphae cells, which produced the decrease of the bioavailable metal concentrations in the immediately vicinity of the root (Denny and Wilkins, 1987 a, b).

On the other hand, it must be highlight that the organic chelatants (inclusive the chelatants released by the microorganisms) were also used for the increase of the plant metal uptake (Salt at al. 1998). In case of fungi the effect of metal uptake increase appear only by very small metal concentration, whenever there is a deficit of metal which function as micronutrients, but not in case of high metal concentration. These findings went to developing of mycoremediation as a much polluted soil remediation technique and to the use of that coupled with phytostabilization.

The aim of this research was to assess the effect of microorganism community structure on the export of metals through percolating water and plant uptake, and to investigate the influence of microorganisms on soil structure and oxidative stress by plants. In this paper are presented selected results from lab and lysimeter experiments.

Materials and methods

The investigated areas were Sorge Settendorf and Gessenhalde, located in the Wismut/Ronneburg uranium mining area from Germany, and Pantelimon located surrounding of the industrial platform "Acumulatorul-Neferal" in the east part of Bucharest city. In the Wismut area more than 220,000 tons of uranium has been mined resulting in the GDR becoming the third-largest uranium producer in the world (Jakubick et al., 2002). Mining started in 1949 and commenced through 1990 when after re-unification the mine was closed and the Wismut GmbH founded for restoration of the area. The former uranium mining site is now an excellent opportunity to study possible mechanisms of heavy metal transfer into water paths and biosphere (Kothe, 2005). Besides the heavy metals, the Gessenhalde is suited also to study the acide mine drainage (AMD). In Pantelimon, approximately 50 ha from surrounding of the two plants are much polluted and require the ecological rehabilitation measures, in accordance with Răuţă et al., 1978; 1980 and Lăcătuşu et al., 2000, sources. The available dates reflect the situation with twelve years ago, thus it is necessary to characterise the actual state of the area from heavy metals contamination point of view. The obtained results together with others, which will be obtained on the experimental way, will be used to the projection of bioremediation plan for the mentioned polluted area.

In frame of more research projects were performed ex-situ lab and lysimeters experiments, the experimental design being different, depending of the proposed objectives. One contaminated soil (0-20 cm) have been used from Pantelimon (brown-red, cernoziom argiloiluvial) and Sorge Settendorf (para brown soil) and due to high soil heterogeneity, three from Gessenhalde in case of pot experiment, and only two: brown-yellow loamy sand (a) and dark red loess loam (b), in lysimeters experiment. The soils a and b (0-50 cm) have been studied in three experimental variants (five replicates each):

mixed with expanded clay without any treatment (control, codified N), mixed with mycorrhizal inoculums (codified M), and mixed with green fertilizer (freshly prepared clover homogenate, codified T).

Five rains occurred during four months in the first experiment, when the contaminated soil had been cultivated with *Lupinus angustifolius*. In the second part of the experiment, when the experimental plant was a rye sp. (*Secale cereale* L.), no strong enough rain occurred in order to produce a leachate.

All soil types were analysed with regard to soil moisture, pH, organic matter, mineral-N concentrations, sulphate, and heavy metals, according to the methods described by Neagoe et al. (2005). After harvesting the plants were weighed (fresh weight) and partitioned for analysis into roots, steams, leaves and pods in case of lupine and only roots and aboveground part, in case of rye. All plant material was frozen and lyophilised for determination of dry weight, ground in an uncontaminated mill and stored at -20^{0}C until processing. All plant analyses are also described in Neagoe et al. (2005). From the leachate the following parameters were analysed: pH, volume, DOC, conductivity and micro and macronutrients.

Results and discussion

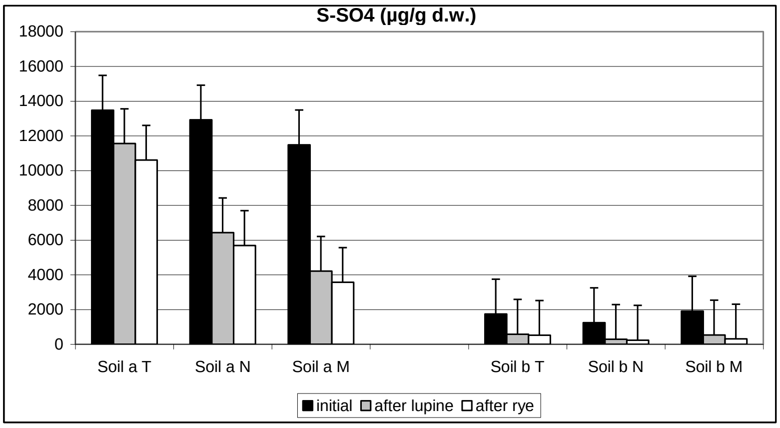
In the Table 1 are presented the selected investigated elements. If the Sorge Settendorf presented extremely high concentration in all investigated elements, in case of Pantelimon the problem consisted especially in very high concentration of lead, followed by zinc and copper. In spite of a relatively lower concentration of elements found in Gessenhalde but a very high heterogeneity of soil, there is a cumulative effect of all pollutants in combination with an extremely dangerous acid mine drainage (AMD).

Tab. 1 Metals in experimental soils

µg/g d.w.		As	Cd	Cu	Pb	U	Zn
Sorge Settendorf	Soil 1	**195**	**14**	**490**	84	**130**	**1124**
Gessenhalde	Soil 2	19	3.2	47	24	UDL	82
	Soil 3	36	2.5	47	24	UDL	69
	Soil 4	72	6.9	52	48	UDL	81
Pantelimon	Soil 5	ND	1.8	221	**1310**	ND	480

Legend: UDL = under detection limit; ND = not determined, bold values = concentration of metals in very high values

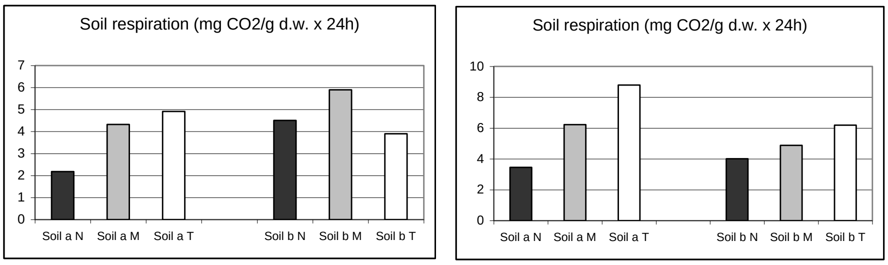
A very high concentration of sulphuric acid was find in so codified soil a (fig. 1) which was classified the most contaminated one from Gessenhalde. Different treatment and plant sp. were applied on soils a, and b. After the two successive culture, first *Lupinus angustifolius* L. and second *Secale cereale* L. the concentration of sulphate in soil decreased significantly in all experimental variants



Legend*:* N-non mycorrhizal variant, M-mycorrhizal variant, T-*Trifolium variant* (green fertilizer)

Fig. 1 Treatment by mycorrhization and green fertilizer

After microorganisms application the soil respiration expressed as a quantity of CO_2 per g d.w. in 24 h, increase in case of soil a and b in both mycorrhizal (M) and *Trifolium* (T) variants after the first and second culture, excepting in soil a, T variant after the first culture. In this experimental variant seems to be a correlation between the high contamination of the fertilized soil and oxic conditions and comparatively better plants development in case of second culture, two month later when the decomposition of clover was completely performed. (fig. 4).



A) B)

Legend: N-non mycorrhizal variant, M-mycorrhizal variant, T-Trifolium variant (green fertilizer)

Fig. 3 Soil respiration from lysimeters cultivated with lupine (A) and rye (B)

The beneficial effect of mycorrhization was quantified through obtaining of a higher concentration of phosphorous and potassium in both plant sp., in underground as well as aboveground part of plants as it can be observed in Figure 5.

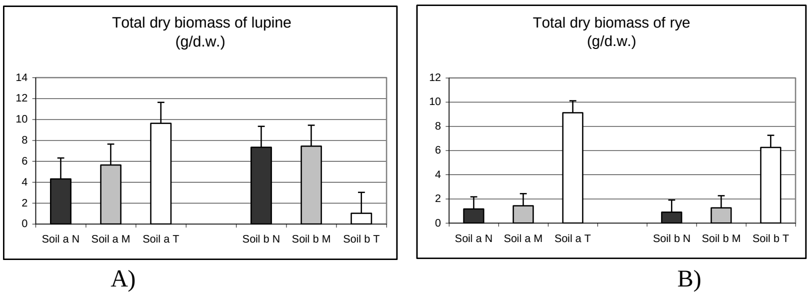


Fig. 4 Total dry biomass of lupine (A) and rye (B); *Legend:* N-non mycorrhizal variant, M-mycorrhizal variant, T-*Trifolium variant* (green fertilizer)

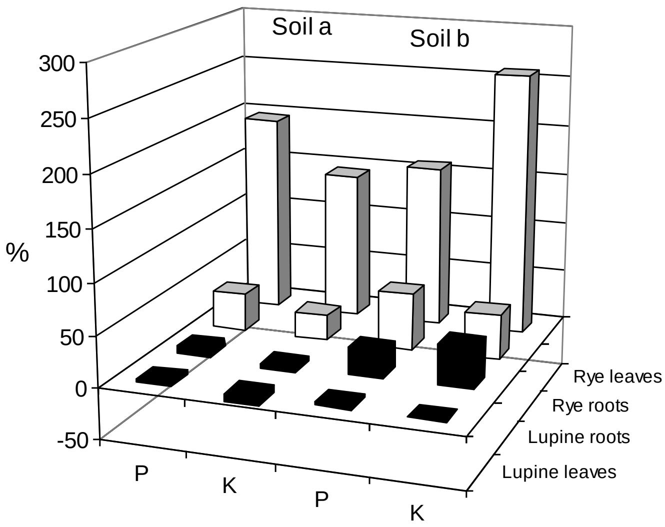


Fig. 5 Variation of nutrients concentration after mycorrhization

From each lysimeter was measured and analysed the water percolated through the soil column, after each abundant hydrological event. In Figure 7 one can remark the influence of mycorrhizal fungi and *Trifolium* amendment on the pH of soil solution (fig. 7A) and some elements export through leachate (fig. 7 B, C and D).

The effect of mycorrhization was also proved by assessing the SOD activity in most plant sp. cultivated on soil from all three investigated area. Figure 6 shows that in all plant sp. the biomass increased and the SOD activity decreased as an effect of mycorrhization. Therefore, it can be concluded that the mycorrhizo-phytoremediation is successful over a large range of soil types, contamination and plant species.

After each hydrological event, the pH measured in the leachate which was possible to be sampled (the water couldn't penetrate each soil column due to the very different soil permeability) increased in all experimental variant, while the concentration of Mg, Al and Mn decreased as a washing effect. Other measured parameters (Neagoe et al., 2008) such as redox conditions, conductivity, DOC or organic matter influenced the water percolation.

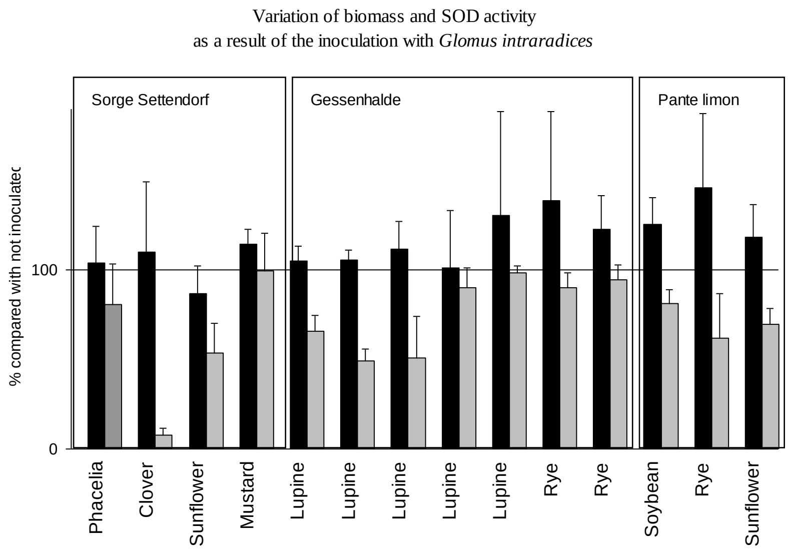


Fig. 6 Variation of biomass (black column) and SOD activity (gray column) as a result of inoculation with *Glomus intraradices*

It was also calculated the total export of all investigated elements by leachate and plants (fig. 8) and the percentage was lower as a result of mycorrhization in case of soil a and higher in case of the most contaminated soil b. In consequence it can be concluded that the soil microorganisms are beneficial effect on leachate in case of soil that is not heavily contaminated with metals.

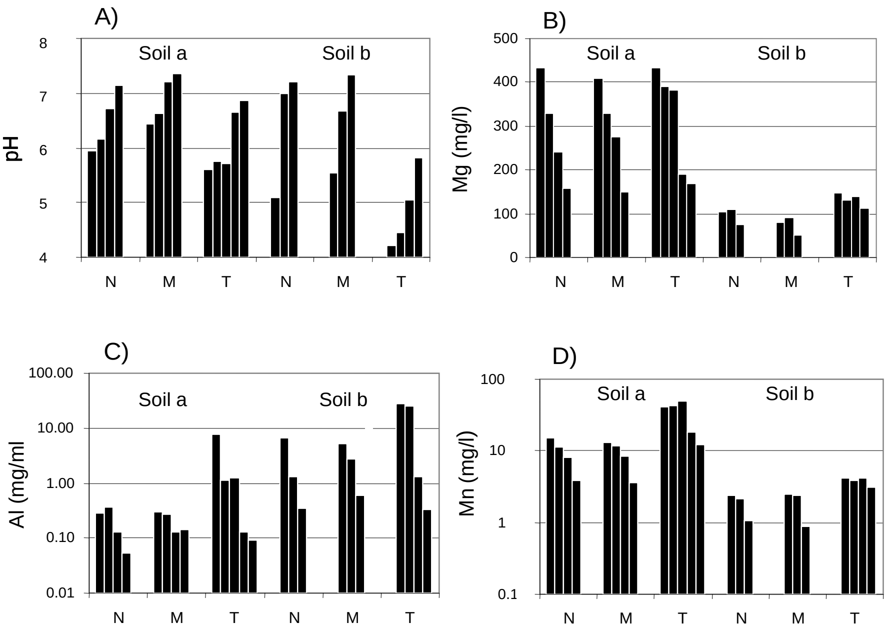


Fig. 7 pH variation and elements concentration in the sampled leachate

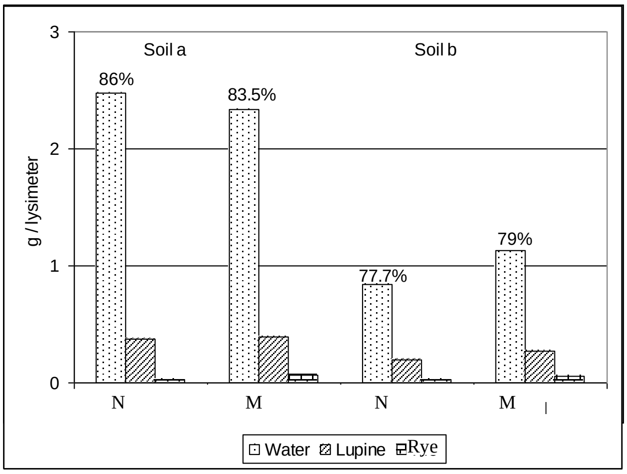


Fig. 8 The export of elements by leachate and plant uptake

Conclusions

The beneficial effect of inoculation with mycorrhizal fungi consisted in the decrease in concentration of some metals in plants, an increase of macro- and micronutrients, particularly phosphorous and potassium, a decrease in the oxidative stress and an increase of the biomass production. It can be also concluded that the heavy metals percolation through soil columns from lysimeters is correlated with the degree of soil contamination, pH, redox conditions, dissolved organic carbon, the previous soil washing, the time of precipitation in correlation with the stage of plants development as well as ecological characteristics of plants and microorganisms (e.g. potential of mycorhization).

Future research direction include the developing of a lysimeter mezocosm experiment in the Pantelimon heavy metals polluted area, including a quantitative assessment of the leachate and simulating the rainfall from the field. Different soil microorganisms will be used and the influence of them on the rate of metals percolation will be assessed.

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